

REMARKS

Claims 1-8 constitute the pending claims in the present application. Applicants cancel, without prejudice, previously withdrawn claims 9-15. Applicants reserve the right to prosecute claims of similar or differing scope in future patent applications. Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

1. Applicants note with appreciation that the amendments put forth on February 6, 2004 have been entered in full.
2. Claims 1-8 are rejected under 35 U.S.C. 112, second paragraph, as indefinite for allegedly failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention. Applicants traverse this rejection and contend that the rejection is moot in light of the amended claims.

Applicants contend that, at the time of filing of the present application, the term "hedgehog polypeptide" was well known and readily understood in the art. In addition to the references cited in the specification and the references cited by the Examiner, Applicants contend that at the time of filing of the instant application well over 1000 papers on the topic had been published. Accordingly, one of skill in the art can readily appreciate the meaning of the term "hedgehog polypeptide" and can readily refer to the literature or to web-based sequence resources such as GenBank to elucidate the nucleic acid or amino acid sequence of a hedgehog polypeptide.

The Office Action directs the Applicants' attention to portions of the specification that allegedly attach a broader meaning to the term "hedgehog polypeptide." The Office Action appears to allege that these portions of the specification make it unclear exactly what is meant by the term "hedgehog polypeptide." Applicants respectfully disagree with the reasoning underlying this rejection. In light of the enormous body of scientific literature relating to hedgehog polypeptides (e.g., sonic, indian, and desert hedgehog) Applicants contend that one of skill in the art would readily appreciate the metes and bounds of the claimed subject matter. Nevertheless, to expedite prosecution, Applicants have amended the claims to more particularly point out that the hedgehog polypeptides for use in the presently claimed methods are hedgehog

polypeptides comprising the sequence of a naturally-occurring hedgehog polypeptide, or N-terminal autoproteolytic fragment thereof. Applicants' amendments are not in acquiescence of the rejection, and Applicants reserve the right to prosecute claims of similar or differing scope.

Furthermore, Applicants contend that one of skill in the art would readily appreciate the meaning of the term "dipalmitoyl hedgehog," as recited in claims 4-7. The prefix "di" is commonly used throughout the scientific and non-scientific literature to denote two. Accordingly, even an untrained reader would interpret the term "dipalmitoyl hedgehog" to mean a hedgehog polypeptide appended with two palmitoyl moieties. However, the threshold for satisfying the requirements of 35 U.S.C. 112, second paragraph, is not whether an untrained reader would understand the metes and bounds of the claimed subject matter, but whether one of skill in the art would appreciate the metes and bounds of the claimed subject matter.

One of skill in the art is not left to interpret the claims based solely on the plain meaning of the prefix "di," one of skill in the art also has the context provided by the specification and the body of scientific literature. These sources further and unambiguously lead to the obvious interpretation of the term "dipalmitoyl hedgehog" to mean a hedgehog polypeptide appended with two palmitoyl moieties. First, we turn our attention to the evidence provided by the specification. In the Summary of the Invention, Applicants outline that the application discloses the use of hydrophobically-modified hedgehog polypeptides, and as a specific example, dipalmitoyl hedgehog (page 5, lines 26-30). Thus, from the beginning of the application, one of skill would clearly appreciate that the term dipalmitoyl hedgehog refers to a particular hydrophobically modified hedgehog polypeptide. This interpretation is further enforced by the definition of "hydrophobically modified hedgehog polypeptide" and "lipophilic moieties" (page 8, lines 3-6; page 9, lines 20-25). Clearly, a palmitoyl moiety is a lipophilic moiety, as defined by the specification, and as generally appreciated in the art. Clearly, the invention contemplates modifying hedgehog polypeptides with one or more moieties. Thus, in light of the clear guidance provided by the specification, one of skill in the art would readily appreciate that the term "dipalmitoyl hedgehog" refers to one example of a hydrophobically modified hedgehog polypeptide. In this case, the example unambiguously refers to a hedgehog polypeptide modified with two palmitoyl moieties.

Second, Applicants note that the terminology employed in the specification to refer to a polypeptide modified with multiple lipophilic moieties was not unique to the present application, and thus one of skill in the art is also guided by the nomenclature used generally throughout the scientific literature. In fact, prior to the time of filing, researchers throughout the world commonly used terms such as “dipalmitoyl” to refer to the modification of a polypeptide or lipid with two palmitoyl moieties. A quick search of the literature uncovered approximately 100 papers, published prior to the filing date of the instant application, that used the term “dipalmitoyl.” By way of example, Applicants enclose herewith the abstracts of a few of these papers (Belsito et al., 2000; Elliott and Prestwich, 2000; Nishijo et al., 2000; Spragg et al., 2000; Ghosh et al., 1990; enclosed herewith as Exhibits 1-5). Without discussing in detail the content of these publications, the abstracts clearly indicate that reference to modified polypeptides or lipid vesicles using terms such as “dipalmitoyl” was readily and unambiguously employed by many researchers in the art. Accordingly, Applicants’ use of similar terminology to refer to the modified hedgehog polypeptides of the present invention readily and unambiguously allows the skilled artisan to appreciate the metes and bounds of the claimed invention.

Applicants do acknowledge that the interchangeable use of the terms “dipalmitoyl hedgehog” and “di-palmitoyl hedgehog” may have introduced unnecessary inconsistency into the claims. Although Applicants contend that the two terms clearly refer to the same modified polypeptide, Applicants have amended the claims to consistently recite “dipalmitoyl hedgehog,” as the term “dipalmitoyl” is unhyphenated in many other publications in the art. Applicants’ amendment is made purely for consistency and does not narrow the scope of the claims.

Applicants contend that recitation of the terms “naturally-occurring hedgehog polypeptide” and “dipalmitoyl hedgehog polypeptide” are clear and unambiguous. One of skill in the art is guided by both the specification, and by an extensive array of publications in the art at the time of filing of the instant application. Accordingly, the metes and bounds of the claimed subject matter are clear, and Applicants respectfully request reconsideration and withdrawal of this rejection.

3. Claims 1, 2, 4, 6, 11 and 12 are rejected under 35 U.S.C. 112, first paragraph, for allegedly failing to enable one of skill in the art to practice the claimed invention. Applicants traverse this rejection and contend that the rejection is moot in light of the amended claims.

Applicants contend that the previously pending claims were enabled throughout their scope. Applicants maintain that the instant specification is broadly enabling, and that one of skill in the art could readily make and test hedgehog polypeptides, fragments, and variants to select suitable compositions for use in the claimed methods. Nevertheless, to expedite prosecution, Applicants have amended the claims to incorporate the features of claim 3 that were previously indicated by the Examiner as constituting enabled subject matter. Applicants' amendments are not in acquiescence to the rejection, and Applicants reserve the right to prosecute claims of similar or differing scope. In light of Applicants' amendments to the claims to incorporate the features of claim 3, reconsideration and withdrawal of this rejection are requested.

Applicants note that claim 3 has been amended to recite that the hedgehog polypeptides of claim 1 are modified with two hydrophobic moieties. Applicants contend that recitation of "two hydrophobic moieties" is supported by the specification, and thus that the amendment to claim 3 does not constitute new matter. Specifically, Applicants direct the Examiner's attention to the definition of "hydrophobically modified hedgehog polypeptide" where the specification clearly contemplates polypeptides modified with one or more moieties (page 8, lines 3-6). Given that two moieties fall squarely within the scope of "one or more," Applicants contend that recitation of "two moieties" is implicitly supported by the specification. However, Applicants need not rely on implicit support alone. The working examples provided in the application include **dipalmitoyl** sonic hedgehog and **dipalmitoyl** Indian hedgehog. Given that the clear meaning of the prefix "di" is two, Applicants contend that working examples of polypeptides modified with two hydrophobic moieties provide explicit support for the amendment to claim 3.

5. Claims 1-3 and 8 are rejected under 35 U.S.C. 103(a) as allegedly obvious in light of US Patent No. 5844079 (Ingham et al.). Applicants traverse this rejection and contend that the rejection is moot in light of the amended claims.

Ingham et al. was previously cited by the Examiner as allegedly anticipating the claimed invention. Now, Ingham et al. is being cited by the Examiner as allegedly rendering the claimed

invention obvious. Applicants respectfully disagree with any allegation that the broad teachings of Ingham et al. undermine the patentability of the presently claimed invention.

Applicants contend that a valid patent may issue for a nonobvious species related to a prior patented invention, even though the improvement falls within the disclosure of that prior patent. A prior genus which **does not explicitly** disclose a species does not anticipate a later claim to that species. This position is well supported by the holdings of the Federal Circuit. See, for example, *Corning Glass Works v. Sumitomo Electric U.S.A.*, 868 F.2d 1251, 1262, 9 USPQ2d 1962, 1970 (Fed. Cir. 1989).

Applicants contend that the relationship between the pending claims and the cited art is largely analogous to the factual situation in the above example. Applicants assert that the presently claimed invention provides a particular combination of elements and constitutes a species. Applicants' species is unobvious and patentable over the generic teachings of Ingham et al. because Ingham et al. fail to either teach or suggest the particular combination of elements recited in the pending claims.

Applicants contend that Ingham et al. fail to teach or suggest all the limitations set forth in the claims. Although Ingham et al. is broadly enabling and provides compositions and methods using *hedgehog* polypeptides, Ingham et al. fail to teach the benefits of the particular combinations of specific hydrophobic modifications, formulations, concentration, and method of use set forth in the pending claims. That is, although Ingham et al. broadly teach methods and compositions using *hedgehog* polypeptides, Ingham et al. provide no motivation to specifically select the particular hydrophobic modifications, the particular formulation, the particular concentration, or the particular method of use, as presently claimed. MPEP 2144.08 outlines the guidelines for determining that a reference renders an invention obvious and directs the Examiner to "determine whether one of ordinary skill in the relevant art would have been motivated to make the claimed invention as a whole, i.e., to select the claimed species or subgenus from the disclosed prior art genus." Applicants contend that Ingham et al. fail to provide motivation to select the specific class of hydrophobic modifications, the particular formulations, the particular concentration of *hedgehog* polypeptide, or the particular method of use. Furthermore, Ingham et al. fail to provide motivation to combine each of these particular

elements, as presently claimed. Although the Office Action alleges that one of skill in the art would eventually arrive at the particular concentrations and formulations recited in the pending claims as part of routine optimization, the Examiner has not provided any evidence or additional references that would have motivated one of skill in the art to choose from amongst the embodiments disclosed by Ingham et al. to select these particular elements and arrive at Applicants' invention. The question of whether one of skill in the art would eventually optimize particular parameters to arrive at limitations recited in the claims is irrelevant if one of skill in the art would have never been motivated to select the particular parameters that require optimization.

Applicants maintain that Ingham et al. fail to satisfy the criteria necessary for rendering obvious Applicants' invention. The MPEP and a substantial body of case law clearly recognize the patentability of a species despite the presence of prior art to the genus, as is the case here. Accordingly, the claimed invention is patentable in light of the prior art, and reconsideration and withdrawal of this rejection is respectfully requested.

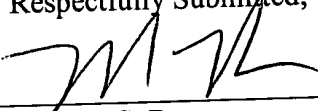
CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945**.

Date: July 27, 2004

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Respectfully Submitted,



Melissa S. Rones
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Molecular and mesoscopic properties of hydrophilic polymer-grafted phospholipids mixed with phosphatidylcholine in aqueous dispersion: interaction of dipalmitoyl N-poly(ethylene glycol)phosphatidylethanolamine with dipalmitoylphosphatidylcholine studied by spectrophotometry and spin-label electron spin resonance.

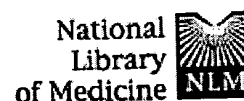
Belsito S, Bartucci R, Montésano G, Marsh D, Sportelli L.

Dipartimento di Fisica and Unità INFM, Università della Calabria, I-87036 Arcavacata di Rende (CS), Italy.

Spin-label electron spin resonance (ESR) spectroscopy, together with optical density measurements, has been used to investigate, at both the molecular and supramolecular levels, the interactions of N-poly(ethylene glycol)-phosphatidylethanolamines (PEG-PE) with phosphatidylcholine (PC) in aqueous dispersions. PEG-PEs are micelle-forming hydrophilic polymer-grafted lipids that are used extensively for steric stabilization of PC liposome to increase their lifetimes in the blood circulation. All lipids had dipalmitoyl (C16:0) chains, and the polymer polar group of the PEG-PE lipids had a mean molecular mass of either 350 or 2000 Da. PC/PEG-PE mixtures were investigated over the entire range of relative compositions. Spin-label ESR was used quantitatively to investigate bilayer-micelle conversion with increasing PEG-PE content by measurements at temperatures for which the bilayer membrane component of the mixture was in the gel phase. Both saturation transfer ESR and optical density measurements were used to obtain information on the dependence of lipid aggregate size on PEG-PE content. It is found that the stable state of lipid aggregation is strongly dependent not only on PEG-PE content but also on the size of the hydrophilic polar group. These biophysical properties may be used for optimized design of sterically stabilized liposomes.

PMID: 10692327 [PubMed - indexed for MEDLINE]

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Maleimide-functionalized lipids that anchor polypeptides to lipid bilayers and membranes.

Elliott JT, Prestwich GD.

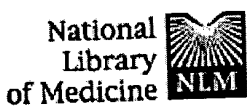


Department of Physiology and Biophysics, The University at Stony Brook, Stony Brook, New York, USA.

Two maleimide-containing diacylglycerol derivatives were synthesized to permit the anchoring of short peptides and longer polypeptides to phospholipid bilayers and membranes. The maleimide was introduced at the site normally occupied by a phospholipid headgroup. The first lipid, the dipalmitoyl ester of 1-maleimido-2,3-propanediol, was developed as a membrane anchor for extracellular domains of transmembrane proteins. The second anchoring lipid, in which the 3-position contained a 6-aminohexanoate, was designed for convenient modification with amine-reactive reporter groups. Specifically, the NBD fluorophore, 7-nitrobenzo-2-oxa-1,3-diazole-aminohexanoic-N-hydroxysuccinimide ester, was attached to give an fluorescent anchoring reagent. Next, these reagents were applied to the anchoring of a C-terminally cysteamine-modified 8 kDa polypeptide that comprises the extracellular N-terminal domain of the human thrombin receptor, a transmembrane protease-activated receptor (PAR-1). Gel filtration and fluorescence analysis showed that the fluorescent lipopolypeptide spontaneously inserted into preformed phospholipid vesicles, but it did not insert into whole cell membranes. In contrast, the dipalmitoyl derivative could only be reconstituted into artificial membranes by mixing the lipopolypeptide and phospholipid before vesicle formation. These results suggest that biophysical interactions governing the lipopolypeptide insertion into artificial and cellular membranes may differ. The thiol-reactive lipidating reagents should be valuable materials for studying the structure and function of peptides and polypeptides at phospholipid bilayer surfaces.

PMID: 11087332 [PubMed - indexed for MEDLINE]

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Interactions of cyclodextrins with dipalmitoyl, distearoyl, and dimyristoyl phosphatidyl choline liposomes. A study by leakage of carboxyfluorescein in inner aqueous phase of unilamellar liposomes.

Nishijo J, Shiota S, Mazima K, Inoue Y, Mizuno H, Yoshida J.

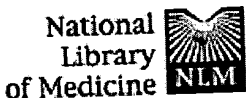


Kobe Pharmaceutical University, Japan.

The interaction of cyclodextrins (CDs) with L-alpha-dipalmitoyl phosphatidyl choline (DPPC), L-alpha-distearoyl phosphatidyl choline (DSPC), and L-alpha-dimyristoyl phosphatidyl choline (DMPC) unilamellar liposomes was investigated by the leakage of carboxyfluorescein (CF) entrapped in the inner aqueous phase of liposomes, at 25 degrees C (DPPC and DSPC liposomes) and at 5 degrees C (DMPC liposomes). The efficiency of CDs for CF leakage was remarkable in the order of heptakis (2,6-di-O-methyl)-beta-CD (DOM-beta-CD) > alpha-CD > heptakis (2,3,6-tri-O-methyl)-beta-CD (TOM-beta-CD) from DPPC liposomes, in the order of DOM-beta-CD > TOM-beta-CD > alpha-CD from DSPC liposomes and in the order of alpha-CD > DOM-beta-CD > TOM-beta-CD from DMPC liposomes. The other CDs used in the present studies, beta-CD, 2-hydroxypropyl beta-CD, and gamma-CD scarcely induced the CF leakage from above the three liposomes. From the profiles of % CF leakage, together with measurements of differential scanning calorimetry, it was found that hydrophobic DOM-beta-CD penetrates the matrix of the liposomes to interact with them as well as TOM-beta-CD, and that less hydrophobic alpha-CD exists at the surface of the membrane to interact with the liposomes. Further, it was found that the interaction of CDs with liposomes changes depending not only on the length of fatty acid chain of phospholipid (condensation force and hydrophobicity) but also the hydrophobicity and the cavity size of CD.

PMID: 10705474 [PubMed - indexed for MEDLINE]

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Effect of recombinant SP-C surfactant in a porcine lavage model of acute lung injury.

Spragg RG, Smith RM, Harris K, Lewis J, Hafner D, Germann P.

Department of Medicine, University of California San Diego School of Medicine and San Diego Veterans Affairs HealthCare System, San Diego, California 92161, USA. rspragg@ucsd.edu

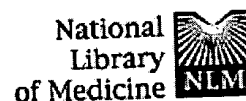
Synthetic surfactants allow examination of the effects of specific components of natural surfactant. To determine whether surfactant containing apoprotein C, dipalmitoyl-phosphatidylcholine, phosphatidylglycerol, and palmitic acid restores gas-exchanging function in acute lung injury (ALI), we administered such surfactant (in doses of 50 or 100 mg/kg and in volumes from 1 to 6 ml/kg) or phospholipid (PL) alone, by intratracheal instillation, to pigs with ALI induced by massive saline lavage. Animals ventilated with 100% O₂ and receiving 1, 2, 4, or 6 ml/kg of 50 mg/kg recombinant surfactant or apoprotein C (rSP-C) surfactant or 2 ml/kg of 50 mg/kg PL (control) had mean arterial PO₂ values, 4 h after treatment, of 230, 332, 130, 142, or 86 Torr, respectively. Animals receiving 1, 2, or 4 ml/kg of 100 mg/kg rSP-C surfactant or 2 ml/kg of 100 mg/kg PL (control) had mean arterial PO₂ values of 197, 214, 148, or 88 Torr, respectively. Surfactant PL distribution was homogeneous. Hyaline membrane formation was reduced in treated animals. Thus, in this model of ALI, rSP-C with PL has the capacity to improve gas exchange and possibly modify lung injury.

PMID: 10658037 [PubMed - indexed for MEDLINE]

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Effect of a fatty acid moiety of phospholipid and ceramide on purified GalT-3 (UDP-Gal:GM2 beta 1-3 galactosyltransferase) activity from embryonic chicken brain.

Ghosh S, Das KK, Daussin F, Basu S.

Department of Chemistry & Biochemistry, University of Notre Dame, Indiana 46556.

Galactosyltransferase, GalT-3 (UDP-Gal:GM2 beta 1-3 galactosyltransferase) has been characterized and solubilized from 19-day-old embryonic chicken brain, and purified to over 2000-fold using mixed-modal chromatography on a omega-aminoethyl Sepharose column and affinity chromatography on a UDP-hexanolamine Sepharose column. The activity of purified GalT-3 was modulated by phospholipids in vitro with stimulation observed specifically with dipalmitoyl phosphatidylethanolamine (PE). All natural phospholipids tested (PE, PC and PI) inhibited GalT-3 activity. Enzyme activity was affected by the structure of the phospholipid vesicle. It was stabilized by the hexagonal (dipalmitoyl PE) structure and inhibited by the bilayer (dioleoyl PE) structure. The long-chain fatty acid moiety of the glycosphingolipid substrate, GM2, was found to be necessary for optimum enzyme activity. In the absence of fatty acid, the modified substrates, lyso-GM2 and acetyl-GM2 had a 10-fold increased K_m and a 4-8 fold decreased V_{max} compared to the normal substrate. We postulate that GalT-3 belongs to a group of glycosyltransferases having recognition for both the carbohydrate as well as the hydrophobic domains (HY-CARS) of their substrates and that the fatty acid moiety of either the substrate (GM2) or a heterotropic effector (phospholipid) plays an important role in regulating the activity of this enzyme.

PMID: 2129344 [PubMed - indexed for MEDLINE]

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